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IN-VITRO ACTIVITY OF THE THIRD GENERATION CEPHALOSPORINS AGAINST CLINICAL ISOLATES RECOVERED FROM WOUNDS

A Thesis Submitted for the Fulfillment of the Requirement for Master

Degree in Microbiology

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بسم الله الرحمن الرحيم

قال تعالى :

(وَمَا أُوتِيتُم مِّن الْعِلْمِ إِلاَّ قَلِيلاً)

صدق الله العظيم

الإسراء الآية(85)

Dedication

To Soul of my

Father and my mother

To my brother and my sisters

To my lively son & husband

To all I love

Sanaa

Acknowledgment

First of all unlimited thanks to Allah for giving me strength and power to complete this study.

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Abstract

This study was carried out in Khartoum state during period between January .2005 to September .2007.

The study evaluates the potency of third generation Cephalosporins

(Ceftazidime, Ceftriaxone, Cefotaxime, Cefoperazone) against clinical isolates recovered from wounds infection caused by bacteria.

Specimens were collected from different hospitals including Khartoum Teaching Hospital, Al Amal National Hospital and National Health Laboratory.

Different types of bacteria were isolated including ***Staphylococcus aureus 55***, ***pseudomonas aeruginosa 45***, ***Escherichia coli 28***, ***Proteus vulgaris 25***, ***Klebsiella Pneumoniae 20***.

The result also indicated that the organisms isolated from males and females were 65% and 35% respectively.

The study showed that Ceftazidime was most effective followed by Cefoperazone, Ceftriaxone and Cefotaxime.

المستخلص

تم تنفيذ هذة الدراسة فى ولاية الخرطوم فى الفترة من يناير 2005 حتى سبتمبر 2007م ، تناولت الدراسة تقييم فاعلية المجموع الثالثة من مضادات السفالوسبورينات (السيفازديم ، السيفترايكسون، السيفوتاكسيم،السيفوبيرازون) لعلاج التهابات الجروح المسببة بواسطة الباكتريا.

تم جمع عينات طبية من عده مستشفيات شملت مستشفى الخرطوم التعليمى ومستشفى الامل الوطنى والمعمل القومى الصحى.

تم عزل وتحديد انواع مختلفه من الباكتريا شملت العنقودية الذهبية 55 سلاله والزائفه الزنجارية 45 سلاله والاشريكية القولونية 28 سلاله والمتقلبه الاعتيادية 25 سلاله والكلبسيلا الرئويه 20 سلاله.

النتائج ايضا اشارت الى ان الميكروبات المعزوله من الذكور هى 65% والاناث 35% على التوالى.

لقد توصلت الدراسه الى ان السيفازديم هو اكثر المضادات الحيوية كفاءة يتبعه السيفوبيرازون، السيفترايكسون واخيرا السيفوتاكسيم.

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List of Abbreviations

NCCLS	National committee for clinical laboratory standards
ATCC	American Type Collection Control
WHO	World Health Organization
SPSS-PC	Social science of personal computer
DNAse	DeoxyriboNucleicease
E.coli	*Escherichia coli*
K	*Klebsiella pneumoniae*
KIA	Kligler-Iron agar
n	Number
Ps	*pseudomonas aeruginosa*
Pr	*Proteus vulgaris*
S	*Staphylococcus aureus*
V.P	Voges –Proskauer
CLED	Cystine-lactose-Electrolyte- Deficient
H_2S	Hydrogen sulphide

Chapter One

1- Introduction and Literature Review

1.1 Infection:

1. 1.1 Definition:

Infection is the mechanism through which microbial agents reach their potential victims and elicit pathogenic reactions (Elegail, 1994). When a microorganism or an infectious agent invades a human or animal body and disease results, whether clinical or sub-clinical, the process is called infection. The microorganism is called a parasite, and the human or animal body is referred to as the host.

The presence of a pathogenic microorganism in or about the body of the host does not alone suffice to produce the interaction referred to as infection.

There for infection is the interaction between a host and a parasite in an environment common to and affecting both

(Infection = host + parasite + environment).

Infection, whether clinically recognizable (apparent) or not recognizable (inapparent or subclinical), cannot occur without a host and a parasite and the environment (Elegail, 1994)

1.1.2 Effects of Infection:

The infections that matter are those causing pathological changes and disease. Many infectious agents cause little or no damage in the host.

Indeed it's of some infections advantage to the microorganism to cause minimal host damage.

When bacteria invade tissues, they almost inevitably cause some damage, and this is also true for fungi, protozoa, and rickettsia. The extent of direct damage however is sometimes slight. This is true for *Treponema pallidum* which produces no potent toxins and attaches to cell in vitro without harmful effects. Leprosy and tubercle bacilli eventually damage and kill the macro-phages in which they replicate, but pathological changes are to a large extent caused by indirect mechanisms (Mims, 1982).

1.1.3 Infectivity:

The infectivity of an organism is its pathogenic potential. This includes successful entry to the body, establishment in the tissues and exertion of its pathogenic effects through invasiveness, toxigenicity or a combination of both (Ross, 1979).

1.1.4 The. Infectious Process:

Infection Indicates multiplication of bacteria. Prior to multiplication, bacteria must enter and establish themselves within the host. Once in the body, bacteria must attach or adhere to host cells, usually epithelial cells. After the bacteria have established a primary site of infection, they multiply and spread.

Infection can spread directly through tissues or via the lymphatic system to the blood stream. Blood stream infection (bacteraemia) can be transient or persistent. Bacteraemia allows bacteria to spread widely in the body and permits them to reach tissues particularly suitable for their multiplication (Jawetz *et al*. 1991).

1.1.5 Sources of Infection:

The terms source, reservoir and vehicle of infection are frequently confused and misused. Source refers to the habitat or growth area in the human or animal. Reservoir and vehicle refer to objects that are contaminated or colonized by microorganisms. Animals are also sources of infection that may be transmitted to man; such infections are known as zoonosis (Ross, 1979).

Infections may be classified according to their sources into:

1. 1.5. 1 Endogenous Infection:

(Autogenous Infections):

Endogenous infections are contracted from the host himself. Many areas of the body have a normal, commensal flora characteristic of the particular area. A most important entity, this flora has many functions, including the provision of a barrier to infection in the individual. Normally the organisms that comprise the commensal flora do not cause infection in the host. There are exceptions to the rule.

There are three common features in endogenous infections:

a) The infections are produced some distance away from the normal habitat of the organisms.

b) Such infections are frequently a manifestation of lowered tissue resistance or tissue damage.

c) Problems of endogenous infections are generally confined to patients, in that they do not generally constitute high cross-infection risks (Ross, 1979).

1. 1.5.2 Exogenous Infections:

Exogenous infections represent the greater proportion of infections and are derived from man, animals or soil. Man is the most common source of exogenous infections, either when the patient is suffering from clinical infection or when the person is a carrier of infection (Ross. 1979).

1.1. 6 Surveillance of Infections:

Surveillance is the key note of infection-control programs. The descriptive epidemiologic data collected by surveillance methods are essential for rational control efforts. Such surveillance data are.

a) Provide baseline rates for detecting increased frequency of infection (outbreaks or clusters).

b) Measure the use and effectiveness of known methods of control.

c) Delineate host procedure and other factors responsible for endemic levels.

d) Provide a basis for evaluating the effect of changes in techniques or equipment.

Potential sources of surveillance data include the hospital record room, the microbiology laboratory; the pharmacy (for review of antibiotic usage) and the wards.

Infections may be tabulated by infection site, agent, ward and service (Clark and Malnan, 1981).

1. 1.7 Transmissibility of Infection:

There are several factors involved in the transmission of infection from the original to the new host. The size of infecting dose is important because successful transmission depends on having sufficient number of organisms present to enter the body, overcome the host resistance and become established in the tissues.

Infections may be classified based on, transmissibility into:

1.1. 7. 1. Localized Infections:

Many infections remain localized and only become generalized:

a) If the organism becomes more virulent.

b) If the host resistance is decreased.

c) If the bacteria gain access to another susceptible part of the body.

1.1.7.2 Generalized Infections:

Some organisms are highly invasive when they enter the body and produce a generalized infection, some are acute and others are sub acute. Spread can be direct by cerebrospinal fluid (CSF), by the blood or by the lymphatic system (Ross, 1979).

1.1.8 Factors That Predispose to Infection:

1. 1. 8.1 Host Factors:

Host-related factors which are likely to predispose to infection include:

a) Age susceptibility to infection is greatest in the very young and elderly. It probably relates to the effectiveness of inflammatory and immune Responses.

b) Genetic factors.

c) The sex of the host.

d) Pre-existing disease.

1.1.8. 2 Environmental Factors:

The most important environmental factors which may predispose to infection include nutrition, climate and animal contact.

1. 1.8. 3 Local Factors:

Some areas of the body have very poor resistance to infections, e.g.; anterior chamber of the eye and joints.

1. 1. 8.4 Other Factors:

These include surgical interference, poor blood supply and presence of foreign bodies (Elegail, 1994).

1.1.9 Types of Infection:

Infections associated with any hospitalization can be divided into two categories (Rayan, 1984):

1. 1. 9.1 Community-acquired Infections:

As defined by the centre for Disease Control (CDC) community-acquired infections are those infections that are present or incubating at

the time of hospital admission, and may be important as they are potentials for spread within the hospitals.

All others are considered nosocomial infections, including those that appear within 14 days of hospital discharge (Ryan, 1984).

1. 1. 9.2 Nosocomial Infections:

A nosocomial infection is an infection that is not present or incubating when a patient is admitted to a hospital. Bacterial nosocomial infections generally have onset' more than 48 to 72 hours after hospital admission, or a nosocomial infection is one acquired during hospitalization (Lennette *et al.,* 1985). Moreover, an infection classified as nosocomial is one in which the patient first becomes symptomatic more than 48 hours after admission, and the incubation period was shorter than the duration of hospitalization or was known (Clark and Malnan, 1981)

In determining whether a given infection is nosocomial or community- acquired, the incubation period of the specified infection, e.g. varicella or hepatitis-B, must be considered. Some nosocomial infections, e.g. surgical wound infections, may have onset after a patient is discharged from the hospital. Such infections are less likely to be recognized by infection control. Personnel infection onset during with than hospitalization (Lennette *et al.,* 1985).

Nosocomial infections may be either endogenous or exogenous (Maki, 1978) endogenous infections are caused by those organisms that are present as part of the normal flora of the patient. Exogenous infections are those caused by organisms acquired by exposure to hospital personnel, or the hospital medical device, environment (Lennette *et al.,* 1985).

Examples of nosocomial infections include:

a) Urinary tract infections in most hospitals between one third and one-half of all nosocomial infections are of the urinary tract and urinary tract infections are the most frequent cause of Gram-negative sepsis.

b) Respiratory infections: pneumonia is the nosocomial infection most often recognized as contributing to death.

c) Postoperative infections: postoperative infections constitute one-third of all nosocomial infections in most institutions. Most wound infections probably come from patient or personnel through direct contact during the' operation or later in recovery rooms or wards.

d) Infections following intravenous therapy: two to eight percent of patients with plastic cannulas in veins over 48 hours develop bacteraemia and non- superlative phlebitis (Clark and Malnan, 1981).

1. 1.9.3 Incidence of Nosocomial Infections:

It has been estimated that approximately 5.5% of hospitalized patients develop nosocomial infection (Dixon, 1978). Combining this incidence with the number of patients admitted annually to hospitals yields an estimate of approximately 2 x^{10} nosocomial infections occurring each year (Bennett, 1978 and Dixon, 1978).

During a 3-year period from 1972 to 1975, the nosocomial infection rate of the University of Virginia Hospital was 6 per 100 admissions or 6% (Wenzel *et al.,* 1976). Also an annual rate of

nosocomial infection in 1980-1982 .ranged from 23.8 to 42.4 per 10,000 patients discharged (Hughes *et al.,* 1983).

The National Nosocomial Infections study (NNIS) is a nation-wide surveillance system organized by, the Centre of Disease Control (CDC) in 1970 in the United States. NNIS represents the only source of current national data on the incidence of and trends in the rate of nosocomial infections (Hughes *et al.,* 1983).

The urinary tract is the most common site of nosocomial infections, accounting for approximately 41% of all nosocomial infections reported by CDC during 1980- 1983. During this period surgical wound infections accounted for 19% of infections, lower respiratory tract infections accounted for 16%, and primary. Bacteraemia accounted for 6%. Infections at all other sites accounted for the remaining 18%, of infections.

Infections rates vary by the type of hospitals (Lennette *et al.,* 1985).

1. 1.9. 4 Modes of Transmission of Nosocomial Infections:

Four modes of transmission of nosocomial pathogens exist (Branchman, 1979). The most common mode of transmission in U.S. is contact transmission, which may result from direct contact between patients or between patients and patent-care personnel. Indirect contact transmission occurs when inanimate objects in the environment, e.g. endoscopes, become contaminated and are not adequately disinfected or sterilized between patients. Droplet transmission, another form of contact spread, occurs by means of large droplets, which can spread over a few feet.

The second most common mode of transmission is common vehicle transmission. Example of contaminated common vehicles implicated in the transmission of nosocomial infections includes food, blood and blood products, diagnostic reagents and medications.

The third most common mode of transmission is airborne transmission. In such instances, infectious agents have been transmitted over great distances. Vector-borne transmission of nosocomial pathogens is rare (Lennette *et at.*, 1985).

1.2. Wound infection

1.2.1. Definition

Wound occurs when break in the continuity of skin or mucus membranes expose the underlying tissue; this may result from accidental injuries, burns, surgical operation and in case of puerperal uterus infection . (vilar,2000).

Infection is mechanism through which microbial agents reach their Potential victims and elicit pathogenic reaction (vilar,2000).

1.2.2. Classification of Wound:

1 .2.2.1. According to Source of Infection in to:

a) Endogenous infection:

Are caused by organisms that have been leading commensal existence elsewhere in Patient's body for example, an abdominal Surgical wound may become inflicted with organisms from the large bowel after an operation involving incision of colon

b) Exogenous infection:

The source of infecting organism is out with body of Patient who Becomes infected cross infection is a particular example of exogenous infection in which the causal organism is spread from person to another .Infection may occur after accidental or Intentional trauma of the skin or other tissue .The latter type is often called "Surgical or post operative sepsis, (Mackie and McCartney, 1996).

1.2.2.2 According to site of infection:

(a) Soft tissue infection: soft tissues are generally associated with the production of Pus and the bacteria involved are said to be pyogenic (Pus –Producing). Not all infection of skin and subcutaneous tissue are supportive in the sense described above (Mackie and McCartney, 1996).

(b) Bone infections: Osteomyelitis and osteitis secondary to a contiguous infections commonly Polymicrobial with aerobic and anaerobic components

(Mackie and McCartney, 1996).

1.2.2.3 According to Surgical Operation:

(a) Clear operation wound: clear surgery that does not involve incision through the gastro intestinal, respiratory, or genitourinary tract the most common causative organism is *Staphylococcus aureus* (vilar,2000).

(b) Contaminated operation wound: Surgery that involves a site with known normal flora apart from the skin, which presents additional risk of contamination of wound. This includes the colon, gall-bladder, mouth and vagina. The causative organisms are gram-negative bacilli and anaerobes such as bactericide fragilis. Dirty (infected) operation wound: The

operation site may occasionally be infected at the time of surgery, as with the incision of an abscess (vilar,2000).

1.2.3 Causitive Agents of Wound Infections:

The type of organism depends on the site and nature of the surgery or trauma. Wound infection following colon, rectal surgery often contains bacteria from large bowel (*E.coli, Bacteroids* spp, etc). Wound infection following bites will contain mouth organisms from the biting animal wound infection are often caused by organism resident on the skin surface which has been breached. The main culprit here is *Staphylococcus aureus* and this is the only constituent of normal skin flora that is worth looking for routinely common: *S.aureus, B.haemolytic, Streptococci, E.coli, Clostridium perfringens,* anaerobic cocci (Manian and Meyer, 1990).

The causative agents of wound infections are *Staphylococci* (30-50%) of all wound infective, *Klebsiella* species, *Pseudomonas aeruginosa, E. coli, Proteus* species, *Bacteroides* species, *Clostridium perfringens, Enterococci,* anaerobic *Streptococci,* anaerobic *Staphylococci* . Wound infections can be caused by *Vibrio vulnifcus* which is halophilic marine vibrio that produced infection in wound exposed to sea -water or raw shell-fish (Elegail,1994). Enteric Gram - negative rod (Enterobacteriaceae) are large hetrogenous group of Gram negative Rod, facultative anaerobes or aerobes, ferment a wide range of carbohydrates, Possess a complex antigenic structure, and Produce a variety of toxins and others virulence factors, these bacteria may also called coli forms (Brooks *et al ;* 2001)

The natural habitat is the intestinal tract of humans and animals (Brooks *et al;* 2001).

1.3 Classification of Entrobacteriaceae:

1.3.1 Classification of Entrobacteriaceae:

The Taxonomy of Enterobacteriaceae is complex and rapidly changing since the Introduction of techniques that measure evolutionary distance, such as nucleic acid Hyperidization and sequencing. More than 25 genera and 110 species or groups have been defined; however, the clinically significant Enterobacteriaceae comprise 20-25 species, and other species are encountered infrequently. The family includes many genera (*Escherichia, Shigella, Salmonella* enterobacter, *Serratia, Proteus* and others. (Brooks *et al;* 2001).

1.3.2 Characteristics:

They are gram-negative rods, either motile with Peritrichous flagella or non motile; they grow on peptone or meat extract media with out the addition of sodium chloride or others supplements; grow well on MacConkey agar, grow aerobically and anacrobically (are facultative anaerobes). Ferment rather than oxidize glucose, often with gas Production; are catalase Positive, oxidase negative, and reduce nitrate to nitrite.

1.3.3 Example of important Pathogens:

1.3.3.1. *Escherichia coli*:

A normal inhabitant in human and animal intestine, grows well as large colonies after overnight incubation. Most strains are motile, some strains are capsulate. Usually, ferments lactose, Indole producer. (Sleigh; 1994).

E. coli is an important cause of sepsis:

a) Urinary tract infection, wound infection especially after surgery of the lower Intestinal Tract, Peritonitis, Biliary Tract infection septicaemia, neonatal meningitis. (Sleigh 1994).

b) Diarrhoea despite being a normal gut commensally, *E-coli* is a common cause of diarrhoea infantile gastroenteritis, tourist diarrhoea and hemorrhagic diarrhoea.

1.3.3.2 *Klebsiella pneumoniae*:

Normal habitat in human and animal intestine. Some strains are saprophytes in Soil, water and vegetation. They Survive well in moist environments in hospital (Sleigh 1994).

Always large and mucoid (due to possession of a prominent capsule) on blood agar, CLED agar and MacConkey agar (Sleigh 1994) *Klebsiella* causes urinary tract infection, septicaemia , meningitis (especially in neonates).Rarely abscess, endocarditic and other lesions .some time associated with chronic nasal and or pharyngeal sepsis and Pneumonia (Sleigh 1994) .

1.3.3.3 *Proteus vulgaris:*

Normal habitat in human and animal intestine .Some strains are saprophytes and are found in soil and water. (Sleigh 1994).

Grow well on routine media. A swarming type of which may cover the whole plate is produced on ordinary media by *Proteus mirabilis* and *Proteus vulgaris. Proteus* forms colourless colonies on MacConkey‘s agar and blue green colonies on CLED agar. (Sleigh 1994).

Proteus mirabilis is main *Proteus* species of medical important. It is a common cause of urinary infection in elderly and young males and often following catheterization or cystoscopy. Infections are also associated with the presence of renal stones.

Abdominal and wound infections, *Proteus* is often secondary invader of ulcer, pressure sores, burns and damaged tissues. Septicaemia and occasionally meningitis and chest infections. *Proteus vulgaris* is occasionally isolated from urine, pus and others specimens. *Proteus mirabilis* infections usually respond better to antimicrobial therapy than those caused by *Proteus vulgaris* (Cheesbrough; 2000).

1.3.3.4 *Pseudomonas aeruginosa:*

Is a Gram-negative bacillus, non sporing, non-capsulate, and usually motile by virtue of one or two polar flagella. It is strict aerobe but can grow anacrobically if nitrate is available. (Green wood *et al., 2002*).

Most saprophytes are found widely in soil, water and others moist environments (Green wood *et al.,* 2002).

The organism grows readily on a wide variety of culture media over a wide temperature range and emits a sweet grape-like odour that is easily recognized. (Green wood *et al*., 2002).

Most strains produce diffusible pigment, typically the colony and surrounding is greenish-blue. The most common colonial form is relative large, low convex with an irregular surface, energy from carbohydrate by an oxidative rather than a fermentative metabolism. All strains give a rapid positive oxidase reaction within 30 seconds (Green wood *et al.*, 2002).

Pseudomonas aeruginosa can infect almost any external sites or organ most community infections are mild and super-facial, but in hospital infections are more commons more sever and more varied (Green wood *et al*., 2002).

1.3.3.5 *Staphylococcus aureus:*

Staphylococcus aureus is the commonest pathogen isolates from subcutaneous abscesses, and skin wounds (Cross infection) common causes of hospital acquired wound infection and ulcer, ostomyelitis and septicaemia.

Staphylococcus aureus is carried in the nose of 40% or more of healthy people (Cheesbrough, 2000).

1.4. Antibiotics

Drugs active against microorganisms are classified in to synthetics and antibiotics (Fadlalla ,2005)

1.4.1. Definition:

Antibiotics are substances produced by microorganisms that inhibit the growth of or kill other, microorganisms. Most antibiotics are secondary metabolites, which are produced at the end of growth phase of microorganisms.Antibiotics have selective toxicity, which enables drugs selectively to kill microorganisms or inhibit its growth while leaving the host, relatively unharmed. (Fadlalla,2005).

1.4.2. Effect:

Antibiotics exert an effect in the patient that is either bactericidal or bacteriostatic. Those antibiotics that are bacteriostatic, e.g. chloramphenicol، erythromycin، tetracycline, inhibit bacterial cell

replication but do not kill the organisms. Other anti biotics, e.g. .penicillins, cephalosporins, aminoglycosides, are bactericidal; they cause microbial cell death, or lysis. A few compounds, e.g. sulfonamides, are either cidal or static according to the composition of the environment (blood, pus, urine, etc) in which the infecting organisms are growing (Pratt, 1977).

1.4.3. Antibiotics Sources:

Most antibiotics are produced by actinomycetes and others produced by fungi and true bacteria.

1.4.4. Antibiotics action:

Antibiotics act in one of two ways either microbiostatically when they inhibit the growth of pathogens and are called bacteriostatic when they act on bacteria e.g. chloramphenicol and tetracyclines or microbicidally when they kill the pathogens and are called bactericidal when act on bacteria e.g.penicillins, cephalosporins, aminoglycosides and sulphonamides.

14.5. Cephalosporins:

The naturally occurring cephalosporins "cephlosporin were first isolated from Cephalosporium fungi in 1945 (Robert and Bryan, 1991).

It is a B-lactam compound with a nucleus 7-aminocephalosporanic acid (Brooks, *et al.*,1995) which consist of B-lactam rings fused to dihydrothiazin ring various substitution at the 3 and 7 position alter their antibacterial activity and pharmacokinetic properties (Albert *et al.*,1991)

1.4.5.1. Mode of Action:

Cephalosporins are bactericidal similar to penicillin. They act by inhibiting synthesis of the bacterial cell wall by binding a bacterial enzyme that is necessary for the formation of celI wall (Eldawa, 1992).

The mechanism of action of cephalosporins is analogous to that of penicillin, binding to specific Penicillin Binding Proteins that serve as drug receptor on bacteria, inhibiting cell wall synthesis by blocking the transpeptidation of peptidoglycan and activating autolysis enzymes in the cell wall that can produce lesions resulting in bacterial death (Brooks *et al.,* 1995) .

1.4.5.2. Pharmacokinetics

Many cephalosporins require parenteral adminisation but a few are available in oral form. Relatively high concentration of these agents are attained across the placenta and in synovial, pleural, and pericardial, bile levels are usually high, especially with cefoperazone which excreted in bile (Albert, *et al.,* 1991).

Cephalosporins are usually excreted in urine unchanged but some form a desacetyl metabolite (Eldawa, 1992). All cephalosporins are excreted primarily by the kidney, except cefoperazone (Albert, *et at.,* 1991).

1.4.5.3. Adverse effects:

Cephalosporins are generally very well tolerated. The most common side effects are diarrhoea and hypersensitivity reaction such as rash, drug fever and serum sickness. Other infrequent effects include pseudo

membranous colitis, elevated serum creatinine and transaminase levels and leucopoenia thrombocytopenia and coombs positive haemolytic anaemia. These abnormalities are usually mild and reversible (Albert, *et aI.,* 1991).

1.4.5.4. Classification:

The most widely used system of classification of cephalosporins is by generation. It is based on the general features of their antibacterial activity and pharmacokinetics characteristic but may depend to some extent on when they were introduced (Eldawa, 1992).

1.4.5.4.1. First Generation:

The first generation are very active against Gram-positive cocci except Enterococci and methicillin resistant *Staphylococcus aureus,* and moderately active against some Gram negative rods, primarily *Escherichia coli, Proteus* spp and *Klebsiella* spp Anaerobic cocci are often sensitive. Bacteriods-fragilis is resistant (Brooks, *et al,* 1995). Have moderate activity against *Haemophilus influenzae*.

First generation Cephalosporins include Cefadroxil, Cefazolin.Cephalexin, Cephaloridine, Cephalothin, Cephapirin, and Cephradine (Albert, *et al.,* 1991).

Cephalexin, Cephradine, and Cefadroxil are absorbed from the gut to a variable extent and can be used to treat urinary and respiratory tract infections. Other first generation cephalosporins must be injected to give adequate level in blood and tissues; Cefazolin is a choice for surgical

prophylaxis because it gives highest 90-120 mg/ml levels. Cephalothin and cephapirin in the same dose give lower level, none of them penetrate the central nervous system and they. Are not drug of choice for any infection (Brooks *et al.,* 1995).

1.4.5.4.2. Second Generation:

The second generation cephalosporins are heterogeneous group, all are active against organism covered by first generation drugs, but have extended coverage against Gram- negative rods including *Klebsiella spp*, *Enterobacter spp* and *Proteus spp* but not *pseudomonas aeruginosa* (Brooks *et al.,* 1995).

Oral second generation cephalosporins can be used to treat sinusitis and otitis media caused by *Haemophilus infuenzae* including *B-Iactamase* producing strains (Brooks *et al.,* 1995).

Cefoxitin and Cefotetan are particularly active against *Bacteriodes fragilis* and thus are used in mixed anaerobic infections including peritonitis or pelvic inflammation. Cefamandale, Cefuroxime, Cefonicid and Ceforanide are injected intravenously at internal in the treatment of Gram-negative bacterial pneumonias or other community acquired infections (Brooks *et al.,* 1995).

1.4.5.4.3. Third Generation

Third generation Cephalosporins have little activity against Gram-positive cocci, *Staphylococci* and *Enterococci.* Often produce super infection during their use. A major advantage of third generation drugs is their enhanced activity against Gram-negative rods. Where as second generation drugs tend to fail against *Pseudomonas aeruginosa,* Ceftazidime or Cefoperazone may succeed. Thus third generation drugs

are very useful in the management of hospital acquired Gram-negative bacteraemia. In immunocompromised patients, these drugs are often combined with aminoglycosides; Ceftazidime may also be lifesaving in severe melioidosis (*pseudomonas pseudomallei* infection). Another important distinguishing feature of several third generations except Cefoperazone is the ability to reach central nervous system and to appear in the spinal fluid in sufficient concentration to treat meningitis caused by Gram-negative rod. Cefotaxime, Ceftriaxone, Ceftazidime are given intravenously and considered the choice for management of gram negative bacterial sepsis and meningitis (Brooks *et al.*, 1995).

Third generation Cephalosporins broad spectra of Gram-negative activity is due to their stability to B-lactamase and their ability to penetrate through the outer cell envelope of Gram-negative bacilli, there are two sub groups among these agents: those with potent activity against *pseudomonas aeruginosa* (Ceftazidime and Cefoperazone) and those without such activity (Ceftizoxime, Cefotaxime, Ceftriaxone and Moxalactam (Awad, 2007).

Cefotaxime inhibits more than 90% of strains of Enterobacteriaceae. It has moderate activity against anaerobes. Ceftazidime, Ceftriaxone and moxalactam have spectra activity similar to that of Cefotaxime with few exceptions. Ceftriaxone is the most active agent against penicillinase positive or negative strain of *Nesseria gonorrhea* (Albert, *et aI.*, 1991). Moxalacam

an oxa B-lactam, is less active against Gram- positive cocci including *Staphylococci* and *Streptococci*. It has slightly more activity

against *Pseudomonas* species than Cefotaxime. It is the most active third generation agent against anaerobes including *Bacteroides -fragilis*.

Cefoperazone is less active than Cefotaxime against many Enterobacteriaceae and Gram-positive cocci. However it has activity against *Pseudomonas aeruginosa*, it is activity against anaerobes is similar to that of Cefotaxime . (Lesch,*et al* ;2001)

Ceftazidime is the most potent of the currently available Cephalosporins against *Pseudomonas aeruginosa*. It is more active than ureidopenicillins against these strains. This agent has activity similar to that of cefotaxime against the Enterobacteriaceae but is not as active against Gram-positive cocci it has little activity against Gram negative anaerobes. Cefixime, the first oral third generation Cephalosporins, has activity against Gram-negative aerobes similar to that of Ceftazidime. It is more stable than other oral Cephalosporins against Gram-negative bacteria. Compared with these agents Cefixime is equally active against *streptococci* but considerably less active against *Staph aureus*. None of the currently available cephalosporins is active against Enterococci (Albert, *et aI, 1991*)

1.4.5.4.4. Fourth Generation

New Cephalosporin's are being classified as fourth generation drugs. The new agents have comparable or slightly enhanced activity against Enterobacteriaceae that are resistant to third generation. They are not active against *Pseudomonas aeruginosa* that are resistant to the third generation. The activity against *Streptococci* and Naficillin susceptible *Staphylococci* is comparable to the third generation compound. Example of fourth generation is Cefepime (Brooks, *et al., 1995*).

1. 5. Microbial Resistance:

The wide spread use of antibiotics lead to the occurrence of drug resistance among microorganisms. The resistances have occurred due to a

change in the nature of genes. (David, 1980). A change in the genome of a bacterial cell may be caused either by a mutation in the chromosome of the cell or result from the acquisition of additional DNA from an external source. DNA may be transferred between bacteria by three mechanisms: transformation, transduction and conjugation... (Green wood *et al.*, 2002).

a- Transformation:

Genetic Transformation occurs by transfer of naked DNA from donor to recipients through the medium. (David, 1980). Most species of bacteria are unable to take up exogenous DNA from the environment. Indeed most bacteria produce nuc1eases that recognize and break down foreign DNA. However, bacteria in some genera, notably *pneumococci*, *Haemophilus influenzae* and certain Bacillus species have been shown to be capable of taking up DNA either extracted artificially or released by lysis from cells of another strain. Cells are competent for transformation only under certain conditions of growth usually in late log phase or during sporulation (Green wood *et al.,* 2002). Transformation is limited to Gram positive bacteria but an exception is *Haemophilus.* (Fadlalla, 2005).

b- Transduction:

Transduction is the process that involves the transfer of small amount of DNA, which is carried, from donor to recipients by specific a bacteriophage. It occurs in both Gram negative and Gram positive bacteria. For example Penicillin resistance in Penicillinase-producing *Staphylococcus* is a result of transduction. In spite of the presence of antibiotics resistance due to transduction, it is not responsible for multiple drug resistance. (Fadlalla, 2005).

c- Conjugation:

Conjugation is the process in which one cell, the donor or male cell makes contact with another, the recipient or female cell and the DNA is transferred directly from the donor into the recipient. Certain types of plasmids carry the genetic information necessary for conjugation to occur. Only cells that contain such a plasmid can act as donors, those lacking a corresponding plasmid act as recipients... (Green wood *et al.*, 2002).Although transfer seems to be confined to gram negative bacteria ,the process allows the passage of any number of genes so that conjugation is responsible for multiple drug resistance .It is now known that the conjugation process is responsible for transfer of extra chromosomal genetic elements called resistance-factors (R- factors). (Fadlalla, 2005).

1.5.1 The Genetic Basis of Antibiotic Resistance:

Genes determine all the properties of microorganisms located either on the chromosome or on plasmids or on lysogenic bacteriophages. With regard to antibiotic resistance it is important to distinguish between intrinsic and acquired resistance... (Green wood *et aI,* 2002)

a - Intrinsic Resistance:

Intrinsic resistance is dependent upon the natural insusceptibility of organisms to a particular drug and will always exist. Intrinsic resistance is usually predictable in a clinical situation and should not pose problems provided that an informed and judicious choice is made of appropriate antimicrobial therapy.

b - Acquired Resistance:

A problem of chemotherapy has been the appearance of resistance to particular drugs in a normally sensitive microbial population. An

organism may lose its sensitivity to an antibiotic during a course of treatment. Once resistance has appeared, the continuing presence of an antibiotic exerts a selective pressure in favour of the resistant organisms. Three main factors affect the frequency of acquired resistance, the first is the amount of antibiotic which is being used, the second is the frequency with which bacteria can undergo spontaneous mutations to become resistant and the last one is the prevalence of plasmids able to , transfer resistance from one bacterium to another.

1.5.2 Control of Antibiotic Resistance:

The major cause of the spread of genes conferring antibiotic resistance is the selection pressure brought about by the increasing use of antibiotics in animal and humans, because antibiotics are used as animal feed supplements and used for treatment of animal populations and human patients. So it is important to minimize the use of antibiotic as much as possible and to reduce the chance of cross- infection. Restriction use and availability of antibiotics could prevent further spread of R plasmid and may reduce their incidence. (Green wood *et al.*, 2002).

1.6. Objectives:

1.6.1 General objective:

The aim of this research is to evaluate the activity of the cephalosporins against organisms isolated from wounds infection.

1.6.2 Specific objective

a - To isolate and identify the organisms from infected wounds.

b- To measure the susceptibility and resistance of the causative agent to different antimicrobial agents.

Chapter Two

2-Materials and Methods

2.1 Study area:

This study was done on specimens collected from Khartoum Teaching Hospital, AL Amal National Hospital and National Health Laboratory during January 2005 – to September 2007.

2.2 Sample size:

Two hundred clinical specimens were collected randomly from males and females, from infected wounds.

2.3 Age Group:-

The patients were divided into 5 age groups as follows.

1 — 15
16 — 30
31 — 45
46 — 60
> 60

2 .4 Materials:

2.4.1 Biological Materials:	
Blood	Veterinary Research Labs Center` (soba)
Plasma	Veterinary Research Labs Center (soba)
2.4.2 Chemicals and Reagents:	
Absolute Ethanol	The British Drug Houses Ltd
Amyl alcohol	The British Drug Houses Ltd
Crystal Violet	The British Drug Houses Ltd
Glucose	Oxoid Ltd., England
Hydrochloric Acid	The British Drug Houses Ltd.
Hydrogen Peroxide	The British Drug Houses Ltd.
Immersion Oil	The British Drug Houses Ltd
Legal's Iodine solution	The British Drug Houses Ltd
Methyl red indicator	The British Drug Houses Ltd
P-Dimethyle-	
Aminobenzoic- Aldehyde	The British Drug Houses Ltd
Safranin Red	The British Drug Houses Ltd .
Savlon	Imperial Chemical Industries Ltd.
Sodium Chloride	Oxoid Ltd. England
Sodium Hydroxide	The British Drug Houses Ltd.
Tetramethyl-p-phenylene-diamine	.
dihydrochloride (oxidase reagent)	The British Drug Houses Ltd
'Urea	. Abbott Ltd. U.K.

2.4.3 Culture Media	
Bacteriological Peptone	Oxoid Ltd ،.England
Blood Agar base	Oxoid Ltd., England
Eosin Methylene Blue agar	Oxoid Ltd., England
Kligler Iron Agar	Oxoid Ltd ،.England
MacConkey Agar	Himedia ،.India
Mueller & Hinton Medium	Oxoid Ltd.,.England
Nutrient Agar	xoid Ltd., England
Nutrient Broth	Oxoid Ltd., England
Peptone Water	Oxoid Ltd., England
Simmon's Citrate Medium	Plasmatec Labs. U.K .
Urea Agar Base	Plasmatec Labs. U.K.

2.4.4 Equipment and Instruments:	
Autoclave	Baird & Tatlock, England
Balance	Adam Equipment Co. England
Colony Counter	Gallenkam P. England
Glass Ware etc….	Griffin & George Ltd. England
	and Kimax ltd . U.S.A'
Hot Air Oven	Memmert,Germany.
Incubator	Baird & Tatlock, England.
Microscope	Olympus, type CH20, Japan.
Microscope slide	Horwell Limited, London, UK.
Imm thick	

Swabs	Medical Disposable Industrial
	Complex (MDIC).Saudi Arabia
Water Bath	Grant Ltd, England.
Wire Loop Holder	Baird & Tatlock, England.

2.5 Experimental work

2.5.1 Collection of Specimens

The specimens were collected from infected wound by using sterile cotton wool swab for wound.

2.5.2 Inoculation

Under aseptic conditions near Bunsen burner, the specimens were inoculated on blood agar and MacConkey‘s agar.

2.5.3 Incubation

The inoculated plates were transferred to the incubator and incubated aerobically at 37°C for 24 hours.

2.5.4 Examination of Growth

The primary culture on MacConkey‘s and blood agar that showed significant growth was examined for fermentation on MacConkey‘s agar and haemolysis on blood agar. The morphological character, size, shape, colour, pigment production, haemolysis, odours were studied.

The resultant growth was subcultured on nutrient agar and stored for further investigation.

2.5.5 Identification

The following techniques were used to identify the isolates:

2.5.5.1 Gram Stain

This was use to differentiate the bacteria into Gram- positive and Gram-negative. During examination of smears bacterial shapes and arrangements were studied, the technique was done as follows:

a- A portion of colony was emulsified in a drop of normal saline in clean slides and then spread evenly to prepare thin smear.

b- The smear was fixed by gentle heat and covered with crystal violet for one minute, then washed rapidly with clean water.

c- Lugol iodine was added for one minute and washed with clean water.

d- Alcohol was added for few seconds and washed rapidly by clean water.

e- The smear was covered with safranine (counter stain) for two minutes and then washed with clean water.

f- The smear was examined microscopically, after air drying, using oil immersion lens. The results were reported.

2.6 Methods:

2.6.1 Isolation of clinical isolates:

The swab was inoculated on sterile blood agar plate and MacConkey agar plat and then streaked by means of a wire loop. The plates were incubated aerobically at 37°C overnight. On MacConkey agar plat, the lactose and non- lactose fermenting colonies were isolated. Subcultures were made on MacConkey's agar and nutrient agar. On blood agar plate the B-haemolytic and alpha- haemolytic and non- haemolytic colonies were isolated. Subcultures were made on blood agar, mannitol salt agar and nutrient agar.

In this study, different types of culture media were used to differentiate many types of bacteria.

(a) MacConkey's agar :

It is a differential/selective medium used for cultivation of Entero bacteria and for differentiation lactose fermenting of non-lactose fermenting Entero bacteria.

(b) **Nutrient agar** :

It is a basic ordinary culinary culture medium used for culturing bacteria at 37°C for 24 hours. All samples were cultured and incubated under aerobic conditions.

(c) Blood agar:

It is used to differentiate organisms that may be Beta haemolytic or Alpha haemolytic or non-haemolytic one.

(d) **Mannitol salt agar**:

It is a selective medium used for isolation of *Staphylococci*

(e) Eosin Methylene Blue agar:

It is a selective media used in identification of *E.coli*.

2.6.2 Purification of the Isolated Colonies

This was done by streaking the organisms on Nutrient agar. Single colonies of purified cultures were further sub cultured on sloping agar incubated aerobically at 37 ºC for 24 hours and then preserved in refrigerator at 4ºC until they were used.

2.6.3 Microscopical Examination of the Clinical Isolates:

All these isolates were subjected to microscopical examination to study their morphology and staining properties using the Gram's staining technique Which involved the following steps; fixation of the isolates then covering with crystal violet (60 sec) and washing with water then covering with iodine (60 sec) and washing then colorization with alcohol and washing then covering with safranin (30- 0 sec) and washing then leaving the slide to dry to be ready for examination by microscope.

2.6.4 Biochemical Tests Adopted for Identification of Bacterial Isolates:

I. Catalase Test:

This test acts as a catalyst in breakdown of hydrogen peroxide to oxygen and water and is carried out by pouring 2-3 ml hydrogen peroxide in test tube, and using sterile wooden stick or glass rod to remove several colonies which were grown on blood free media then immerse in hydrogen peroxide solution. Bubbles of oxygen are released if the organism is catalase producer. It is used to differentiate bacteria that produce a catalase enzyme such as *Staphylococci* from non- catalase producer e.g. *Streptococci.* (Cheesbrough, 2000).

II. Citrate Utilization Test:

This test is based on the ability of an organism to use citrate as the only source of carbon and ammonia as the only source of nitrogen. The test organism was inoculated in a medium, which contains sodium citrate, ammonium salt and the Bromothymol blue as indicator or (Simmons citrate medium which is a modification of Kosers medium with agar and

for up to four days. Citrate Utilization an indicator) then incubated at 37c appeared by a change in colour of indicator from light green to blue due to alkaline reaction.

III. Coagulase Test:

Coagulase causes clot of plasma by converting fibrinogen to fibrin. It is carried out by pouring 0.2 m1 of plasma in test tube then adding 0.8 m1 of test broth culture and incubating at 37°c then examining for clot after 1 hour, 3 hours and over night. It is used to identify *Staph. aureus,* which is coagulase enzyme producer. (Cheesbrough, 2000).

IV. Deoxyribonuciease (DNAse) Test:

The test is used to demonstrate the DNase producing organisms and is carried out by culturing an organism in a medium containing DNA and incubating at 37°c over night .The colonies are tested for DNAse production by flooding the plate with a weak hydrochloric acid solution, the acid precipitates unhydrolized DNA therefore clear zone surround DNase producing colonies. It is used to differentiate *Staph. aureus* from other *Staphylococci.* (Cheesbrough, 2000).

V. Indole Test:

This test demonstrates the ability of certain bacteria such as *E.coli* to break down the amino acid tryptophane with the releasing of indole, which accumulates in the medium. The test organism was cultured on a suitable tryptophane rich medium, mainly peptone water for 48 hours, then 0.5m1 of Kovac's reagent is added, a ring of red colour in the alcohol layer indicates a positive reaction. (Cheesbroug, 1984).

VI. Kligler- Iron Agar:

It is a differential slope medium, fermentation of lactose and glucose; production of hydrogen sulphide and production of gas are reactions used in identification. A yellow butt and red slope indicate the fermentation of glucose only. A yellow slope and a yellow butt indicate the fermentation of glucose and lactose. A red slope and a red butt indicate no fermentation of glucose and of lactose. Cracks and bubbles indicate gas production. Blackening indicate hydrogen sulphide production.

VII. Methyl Red Test:

The methyl red test is employed to detect the production of sufficient acid during the fermentation of glucose by change in the colour of methyl red indicator. Inoculate from young aged culture of test organism in buffered glucose peptone water, incubate at 37℃ for 48hour then add five drops of methyl red indicator. A positive reaction is indicated by a red colour and negative by yellow (Cruickshanck *et al.*, 1975)

VIII. Oxidase Test:

This test depends on the presence of oxidase enzyme, in certain bacteria, that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye (tetramethyl-P-phenylene-diamine dihydrochloride) (Cruickshanck *et al.*, 1975). A piece of filter paper is soaked with a few drops of oxidase reagent (tetramethyl-P-phenylene-diamine dihydrochloride). A colony of the test organism is then smeared on the filter paper. If the organism is oxidase - producer, the phenylene diamine in the reagent will be oxidized to a deep purple colour.

IX. UreaseTest:

This test was carried to assess urease enzyme activity, and it is important in recognizing Enterobacteria. The test is carried out by inoculating slopes of medium, which contains urea, with test organism and incubating at 37°C for up to five days and examined daily. If the strain is urease producer, the enzymes will breakdown the urea to give ammonia and carbon dioxide. The releasing of ammonia makes the medium alkaline so the colour of the indicator (Phenol Red) is changed to red-pink (Cheesbroug, 1984).

X. Voges - Proskauer (VP):

Many bacteria ferment carbohydrate with the production of acetyl methyl carbinol. The test is carried by culturing test organism in glucose phosphate peptone water for 48 hours. Sodium hydroxide and small amounts of creatinine were added. Under alkaline conditions and exposure to the air, a pink colour develops within 2-5 minutes which becomes crimson in 30 minutes indicating a positive reaction for acetone production.

2.7 Antimicrobial Susceptibility Testing:

2.7.1.Antimicrobial Susceptibility Testing:

The Kirby-Bauer method of antibiotic susceptibility was used in this study.

2.7.1.1. Materials

2.7.1.1.1. Medium: Mueller Hinton from Oxoid (U.K.) were used

2.7.1.1.2 McFarland ‘s Turbidity Standard

The standard has the turbidity of suspension of approximately 1.5×10^8 bacteria/ml.

Swabs were purchased or local prepared

2.7.2. Procedure For The Disk Agar Diffusion Method:

I. Preparation of Plates:

Twenty ml of sterile Mueller and Hinton Sensitivity Agar medium was poured into sterile plates. After medium has solidified, the plates were dried for 30 minutes in an incubator at 37°C to remove excess moisture from the surface.

II. Preparation of Inoculums and Inoculations:

This preparation was done by emulsifying 2 - 3 colonies of the test organism in a small amount (about 3 - 5ml) of sterile normal saline or nutrient broth. In order to prevent further growth, the diluted and standardized inoculums should not be allowed to stand longer than 15 - 20 minutes before the plates are inoculated. A sterile cotton swab is dipped into a suitable diluted culture or suspension and rotated; the swab is turned against the side of the bottle to remove excess fluid, and is streaked across the medium (David, 1980).

III. Disk application: By using a pair of sterile forceps, antimicrobial discs (ceftazidime, cefoperazone, cefotaxime and ceftriaxone) were placed into each plate for Gram-positive and Gram-negative.

IV. Incubation: Within 15 minutes of preparation all plates were incubated aerobically at 35° C overnight.

V. Reading: after overnight incubation, diameter of each zone of inhibition was measured and recorded to the nearest millimetre. The measurements were made with a ruler on the under surface of the plate without opening the lid.

VI. Result: Result of reading was interpreted according to NCCLS Zone Diameter Interpretive Standards.

2.8. Antimicrobial Discs

The following antimicrobial discs were used in the research:

a). Ceftazidime (CAZ) 30mg , Abtek Biological Ltd.,UK

b). Cefoperazone (CFP) 30 mg Oxoid, UK

c). Ceftotaxime (CTX) 30 mg, Abtek Biological Ltd, UK

d). Ceftriaxone (CRO) 30 mg Bioanalyse , UK

2.9. Control Organisms:

Which include *Escherichia coli* (ATCC 25922) to control Gram-negative bacilli, *Staphylococcus aureus* (ATCC 25923) to control Gram-positive cocci and *Pseudomonas aeruginosa* (ATCC 27853), the disk placed individually with sterile forceps and then gently pressed down on the Mueller-Hinton medium without blood or other supplements. In general not more than 4 disks were placed on plate; (WHO, 1997). Results of reading were interpreted according to the diameters given in the most recant NCCLS documents. (National Committee for Clinical Laboratory Standards).

2.10 Method of data analysis:

The data collected was analyzed using statistical package for Social Science for Personal Computer (SPSS/PC).

3- Results

During the period from January. 2005 – to September. 2007, Two hundred wounds swab were collected randomly from Patients attending(out-pateints) Khartoum Teaching Hospital, National Health laboratory and Alamal National Hospital were Females 70 and Males 130.

Bacteriological test showed 173 specimen positive culture " significant growth " , 27 no growth from the total positive culture only 123 gram negative rods according to Biochemical test 50 were Gram - positive .

Table 1: Distribution specimens according to sex

Sex	Frequency	Percentage
Males	130	65 %
Females	70	35 %
Total	200	100 %

Table 2: Distribution of specimens according to Growth and No Growth

Total	Growth		No Growth	
	Males	**Females**	**Males**	**Females**
200	123	50	7	20

Table 3: Distribution of the five age groups

Age Group	Frequency	Percentage
1 − 15	30	15%
16 − 30	70	35%
31 − 45	50	25%
46 − 60	30	15%
More than 60	20	10%
Total	200	100%

Table 4: Distribution of wound Infection according to pathogen

Pathogen	Frequency	Percentage
Staph aureus	55	31.8%
Pseudomonas aeruginosa	45	26 %
Escherichia coli	28	16.2 %
Proteus vulgaris	25	14.5%
Klebsiella pneumoniae	20	11.6 %
Total	173	100 %

Table 5: Distribution of wound infection according to the age

Age Group	Frequency	Percentage
1 − 15	31	18 %
16 − 30	40	23 %
31 − 45	50	29 %
46 − 60	31	18 %
> 60	21	12 %
Total	173	100 %

3.1 Identification of *Staphylococcus aureus*:

3.1.1 Cultural Characteristics:

On nutrient agar, golden yellow colonies were observed. On mannitol salt agar, it changed the colour of medium from red to yellow.

3.1.2 Microscopical Examination:

With Gram's staining technique, Gram-positive cocci arranged in grape –like clusters.

3.1.3 Biochemical Reactions :

Most of the isolates fermented lactose with production of acid and didn't form gas. All of them fermented sucrose and mannitol with acid production. Most of them fermented glucose with acid production.

Almost all of isolates were Catalase-positive, Coagulase-positive, and DNA se positive.

The production of coagulase identifies staphylococcus aureus, and also DNAse test is a confirmatory test.

3.2 Identification of *Escherichia coli*:

3.2.1 Culture characteristics:

On MacConkey agar medium, large red colonies were observed as a result of lactose fermentation.

3.2.2 Microscopical Examination:

With Gram's staining technique Gram-negative rods were seen.

3.2.3 Biochemical Reactions:

All the isolates fermented lactose with production of acid and gas. Most of them fermented glucose and sucrose with acid production and gas formation.

All the isolates give indole -positive result. All of them were Methyl red-positive and Voges-Proskauer negative. None of the isolates produced urease, utilized citrate or give positive Oxidase.

All isolates did not change the yellow colour of K.I A both in slope and butt, with the absence of H_2S production, but all isolates produced gas.

3.3 Identification of *pseudomonas aeruginosa*:

3.3.1 Cultural Characteristics:

On MacConkey‘s agar .discrete pale colonies were observed. On nutrient agar most of the isolates produced blue -green pigment which diffused in surrounding medium.

3.3.2 Microscopical Examination:

With Gram’s staining technique, Gram-negative rods were seen.

3.3.3 Biochemical Reactions:

Regarding K.I A, all of them changed the slope colour to red, and most change the butt to red with out H_2S or gas production.

Pseudomonas aeruginosa is non-lactose fermenting .All isolates fermented glucose and none of them fermented sucrose. All isolates were Oxidase-positive, indole-negative، Methyl red and Voges – Proskauer negative.

3.4 Identification of *Klebsiella pneumoniae* :

3.4.1 Cultural characteristics:

On MacConkey‘s agar medium, were seen as large pink and mucoid colonies.

3.4.2 Microscopical Examination:

With Grams staining technique, Gram-negative capsulated rods were seen.

3.4.3 Biochemical Reaction:

All isolates were lactose fermenting with acid and gas production Most of them ferment glucose and sucrose with acid or gas production.

All isolates were Indole-negative, Methyl red-positive, and Voges - Proskauer negative. Almost all of them were Citrate-positive.

Klebsiella Pneumoniae gives a positive urease test after 18-24 hours and positive citrate.

3.5 Identification of *Proteus vulgaris*

3.5.1 Cultural Characteristics:

On nutrient agar , fishy smell and swarming appearance, was seen On MacConky‘s agar and deoxycholate citrate agar, pale coloured colonies were observed as a sign of non lactose ferment and with no swarming.

3.5.2 Microscopical Examination:

With Gram’s staining technique, Gram- negative was seen.

3.5.3 Biochemical reactions:

All isolates were non lactose fomenters. Most of the isolates fermented sucrose with acid production and some of them formed gas .All isolates fermented glucose with acid production.

Most of isolates were Methyl red positive, and all of them were Voges -Proskauer negative. Almost all of them were urease positive.

All isolates changed the colour of slope of K.I.A from yellow to red and maintained the yellow colour of butt. Most were H_2S producers and gas non- producers.

3.6 Susceptibility Test:

The interpretation of the inhibition zone diameters was based on the following data (NCCLS).

Table 6:

Activity of Antibiotic	Activity of Antibiotics			
	Ceftazidime	**Cefoperazone**	**Cefotaxime**	**Ceftriaxone**
Resistant	≤ 14mm	≤ 14mm	≤ 14mm	≤ 13mm
Sensitive	≥ 18mm	≥ 23mm	≥ 21mm	≥ 21mm
Intermediate	15 – 17mm	15 – 22mm	15 – 20mm	14 – 20mm

3.7 Quality Control:

Diameters of zone of inhibition (mm) obtained from susceptibility testing of organism's standard strains against antimicrobials of the study which agreed with that on Nccls and that emphasized the validity of the antimicrobial disc

Table 7: Quality Control:

Name of Organisms	Zone of Inhibition			
	Ceftazidime	**Cefoperazone**	**Cefotaxime**	**Ceftriaxone**
Staph aureus	——	26	27	25
Pseudomonas aeruginosa	26	23	21	22
Escherichia coli	30	29	32	33
Proteus vulgarise	25	27	25	30
Klebsiella pneumonia	23	25	30	33

Table8: Diameter of Zones Inhibition of Antimicrobial (mm) Against *Staph aureus*

Isolate Code	Zone of Inhibition			
	Ceftazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
1 S	18 (S)	22 (S)	21 (S)	14 (R)
2 S	14 (R)	14 (R)	21 (S)	14 (R)
3 S	15 (I)	14 (R)	10 (R)	23 (S)
4 S	14 (R)	21 (S)	26 (S)	15 (I)
5 S	00 (R)	00 (R)	21 (S)	23 (S)
6 S	20 (S)	00 (R)	10 (R)	23 (S)
7 S	22 (S)	00 (R)	15 (I)	23 (S)
8 S	00 (R)	26 (S)	00 (R)	23 (S)
9 S	11 (R)	00 (R)	22 (S)	14 (R)
10 S	14 (R)	00 (R)	18 (I)	14 (R)
11 S	13 (R)	11 (R)	14 (I)	15 (I)
12 S	13 (R)	12 (R)	16 (I)	25 (S)
13 S	22 (S)	25 (S)	22 (S)	23 (S)
14 S	00 (R)	25 (S)	11 (R)	12 (R)
15 S	12 (R)	14 (R)	21 (S)	12 (R)
16 S	15 (I)	16 (I)	16 (I)	23 (S)
17 S	17 (I)	16 (I)	16 (I)	15 (I)
18 S	15 (I)	14 (R)	13 (R)	13 (R)
19 S	14 (I)	23 (S)	27 (S)	14 (R)
20 S	00 (R)	23 (S)	28 (S)	16 (I)
21 S	16 (I)	23 (S)	21 (S)	14 (R)
22 S	00 (R)	13 (R)	13 (R)	17 (I)
23 S	13 (R)	12 (R)	13 (R)	26 (S)

Isolate Code	Zone of Inhibition			
	Ceftazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
24 S	16 (I)	12 (R)	23 (S)	26 (S)
25 S	14 (R)	21 (S)	8 (R)	26 (S)
26 S	16 (I)	12 (R)	21 (S)	23 (S)
27 S	13 (R)	10 (R)	10 (R)	15 (I)
28 S	15 (I)	21 (S)	26 (S)	00 (R)
29 S	12 (R)	15 (I)	10 (R)	15 (I)
30 S	14 (R)	15 (I)	26 (S)	00 (R)
31 S	19 (S)	23 (S)	25 (S)	00 (R)
32 S	26 (S)	14 (R)	28 (S)	26 (S)
33 S	11 (R)	21 (S)	12 (R)	23 (S)
34 S	10 (R)	13 (R)	10 (R)	28 (S)
35 S	00 (R)	22 (S)	15 (I)	00 (R)
36 S	14 (R)	13 (R)	15 (I)	11 (R)
37 S	00 (R)	20 (I)	12 (R)	00 (R)
38 S	12 (R)	00 (R)	21 (S)	14 (R)
39 S	12 (R)	24 (S)	20 (I)	28 (S)
40 S	00 (R)	00 (R)	21 (S)	14 (R)
41 S	00 (R)	15 (I)	12 (R)	10 (R)
42 S	22 (S)	13 (R)	24 (S)	14 (R)
43 S	13 (R)	21 (S)	12 (R)	12 (R)
44 S	13 (R)	13 (R)	24 (S)	12 (R)
45 S	13 (R)	13 (R)	12 (R)	11 (R)
46 S	11 (R)	17 (I)	24 (S)	13 (I)
47 S	00 (R)	10 (R)	21 (S)	14 (R)
48 S	00 (R)	10 (R)	11 (R)	14 (R)
49 S	00 (R)	23 (S)	9 (R)	13 (I)

Isolate Code	Zone of Inhibition			
	Ceftazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
50 S	00 (R)	13 (R)	22 (S)	23 (S)
51 S	00 (R)	13 (R)	9 (R)	26 (S)
52 S	14 (R)	22 (S)	22 (S)	23 (S)
53 S	12 (R)	00 (R)	13 (R)	28 (S)
54 S	00 (R)	00 (R)	13 (R)	24 (S)
55 S	00 (R)	00 (R)	20 (I)	24 (S)

Key:

S: Sensitive

R: Resistant

I: Intermediate

Table 9: Susceptibility of *Staph aureus* n (55) to

Activity of Antibiotic	Activity of Antibiotics			
	Ceftazidime	**Cefoperazone**	**Cefotaxime**	**Ceftriaxone**
Resistant	40 (73%)	31 (56%)	20 (36%)	24 (44%)
Sensitive	7 (13%)	17 (31%)	24 (44%)	22 (40%)
Intermediate	8 (14%)	7 (13%)	11 (20%)	9 (16%)
Total	55 (100%)	55 (100%)	55 (100%)	55 (100%)

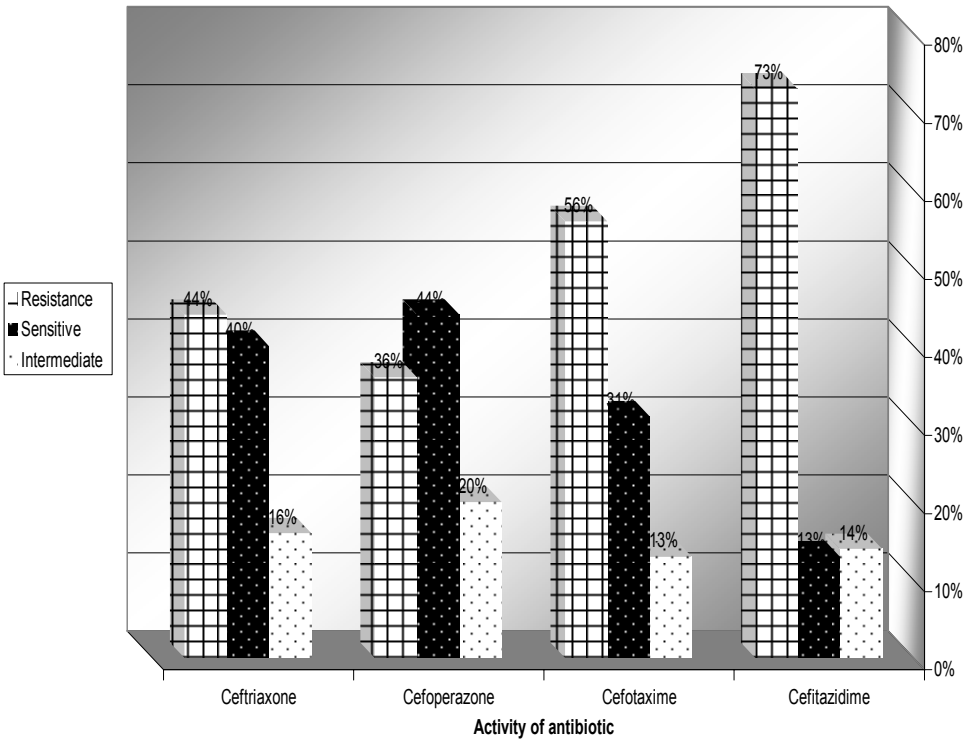

Figure (1): Susceptibility of *Staph aureus* n (55) to Ceftazidime, Cefoperazone, Cefotaxime & Ceftriaxone

Table 10: Diameter of zones inhibition of antimicrobial (mm) against *Proteus Vulgaris*

Isolate Code	Zone of inhibition			
	Ceftazidime	Cefotaxime	Cefoperazone	Ceftriaxone
1 Pr	18 (S)	00 (R)	22 (S)	18 (I)
2 Pr	18 (S)	13 (R)	21 (S)	00 (R)
3 Pr	20 (S)	10 (R)	24 (S)	12 (R)
4 Pr	14 (R)	00 (R)	26 (S)	13 (R)
5 Pr	22 (S)	14 (R)	24 (S)	22 (S)
6 Pr	22 (S)	00 (R)	00 (R)	15 (I)
7 Pr	13 (R)	16 (I)	16 (I)	26 (S)
8 Pr	23 (S)	24 (S)	13 (R)	28 (S)
9 Pr	23 (S)	24 (S)	29 (S)	30 (S)
10 Pr	19 (S)	13 (R)	12 (R)	25 (S)
11 Pr	18 (S)	11 (R)	00 (R)	12 (R)
12 Pr	22 (S)	11 (R)	25 (S)	00 (R)
13 Pr	21 (S)	12 (R)	11 (R)	13 (R)
14 Pr	12 (R)	15 (I)	24 (S)	14 (I)
15 Pr	17 (I)	26 (S)	28 (S)	32 (S)
16 Pr	17 (I)	25 (S)	00 (R)	16 (I)
17 Pr	22 (S)	00 (R)	10 (R)	24 (S)
18 Pr	22 (S)	11 (R)	00 (R)	20 (I)
19 Pr	16 (I)	18 (I)	25 (S)	23 (S)
20 Pr	11 (R)	13 (R)	14 (R)	00 (R)
21 Pr	11 (R)	26 (S)	21 (S)	10 (R)
22 Pr	16 (I)	00 (R)	11 (R)	11 (R)
23 Pr	20 (S)	19 (I)	29 (S)	00 (R)
24 Pr	22 (S)	10 (R)	25 (S)	00 (R)

25 Pr	20 (S)	23 (S)	24 (S)	10 (R)

Key:

S: Sensitive

R: Resistant

I: Intermediate

Table 11: Susceptibility of *Proteus Vulgaris* n (25) to

Activity of Antibiotic	Activity of Antibiotic			
	Ceftazidime	**Cefoperazone**	**Cefotaxime**	**Ceftriaxone**
Resistance	5 (20%)	10 (40%)	15 (60%)	12 (48%)
Sensitive	16 (64%)	14 (56%)	6 (24%)	8 (32%)
Intermediate	4 (16%)	1 (4%)	4 (16%)	5 (20%)
Total	25 (100%)	25 (100%)	25 (100%)	25 (100%)

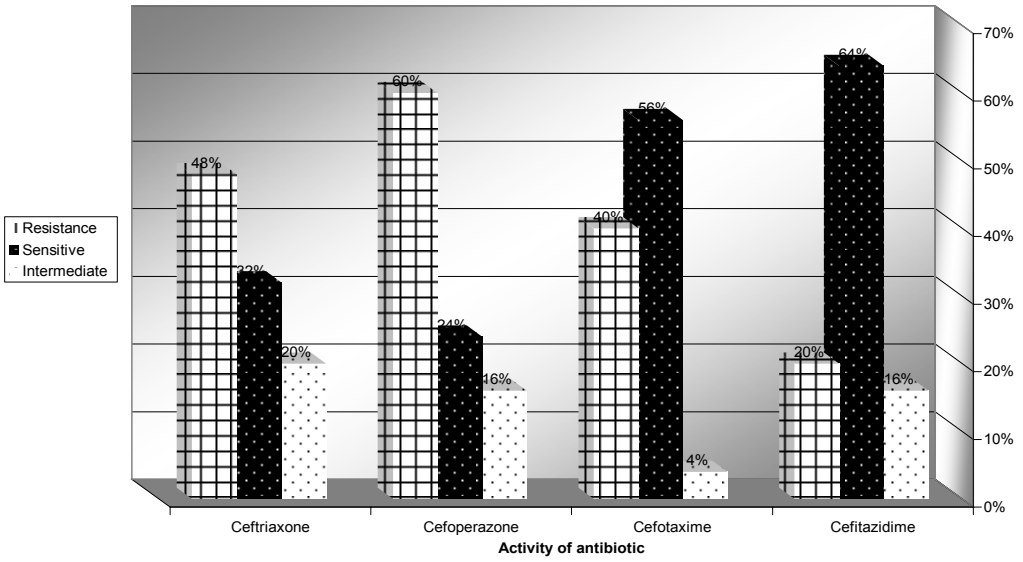

Figure (2):Susceptibility of *Pr* n (25) to Ceftazidime, Cefoperazone, Cefotaxime & Ceftriaxone

Table 12: Diameter of zones inhibition of antimicrobial (mm) against *Klebsiella Pneumoniae*

Isolate Code	Zone of Inhibition			
	Cefitazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
1 K	18 (S)	14 (R)	22 (S)	16 (I)
2 K	18 (S)	12 (R)	26 (S)	14 (I)
3 K	20 (S)	00 (R)	24 (S)	22 (S)
4 K	22 (S)	00 (R)	13 (R)	26 (S)
5 K	23 (R)	11 (R)	00 (R)	26 (S)
6 K	17 (I)	10 (R)	11 (R)	23 (S)
7 K	15 (I)	9 (R)	32 (S)	00 (R)
8 K	18 (S)	25 (S)	30 (S)	12 (R)
9 K	20 (S)	24 (S)	15 (I)	18 (I)
10 K	21 (S)	30 (S)	24 (S)	29 (S)
Isolate Code	**Zone of Inhibition**			
	Cefitazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
12 K	11 (R)	13 (R)	22 (S)	25 (S)
13 K	19 (S)	33 (S)	22 (S)	11 (R)
14 K	22 (S)	30 (S)	00 (R)	10 (R)
15 K	20 (S)	00 (R)	23 (S)	00 (R)
16 K	18 (S)	8 (R)	18 (I)	28 (S)
17 K	22 (S)	29 (S)	23 (S)	26 (S)
18 K	19 (S)	20 (I)	22 (S)	8 (R)

19 K	19 (S)	18 (I)	30 (S)	30 (S)
20 K	21 (S)	24 (S)	00 (R)	30 (S)

Key:

S: Sensitive

R: Resistant

I: Intermediate

Table 13: Susceptibility of *Klebsiella Pneumoniae* n (20) to

Activity of Antibiotic	Activity of Antibiotic			
	Ceftazidime	**Cefoperazone**	**Cefotaxime**	**Ceftriaxone**
Resistance	3 (15%)	5 (25%)	10 (50%)	6 (30%)
Sensitive	15 (75%)	13 (65%)	7 (35%)	11 (55%)
Intermediate	2 (10%)	2 (10%)	3 (15%)	3 (15%)
Total	20 (100%)	20 (100%)	20 (100%)	20 (100%)

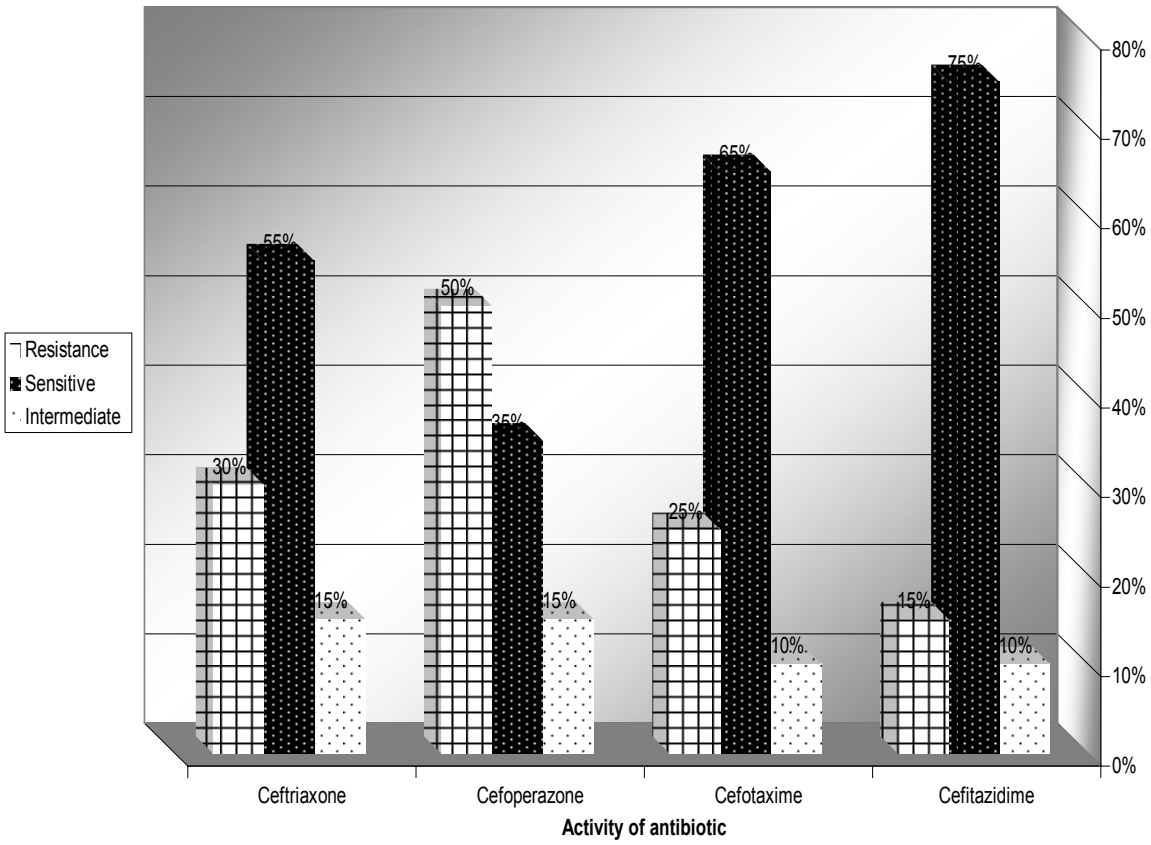

Figure (3): Susceptibility of *Kleb* n (20) to Ceftazidime, Cefoperazone, Cefotaxime & Ceftriaxone

Table 14: Diameter of zones inhibition of antimicrobial (mm) against *Escherichia Coli*

Isolate Code	Zone of Inhibition			
	Ceftazidime	Cefotaxime	Cefoperazone	Ceftriaxone
1 E	22 (S)	13 (R)	23 (S)	22 (S)
2 E	20 (S)	12 (R)	21 (S)	25 (S)
3 E	13 (R)	13 (R)	21 (S)	23 (S)
4 E	13 (R)	29 (S)	22 (S)	00 (R)
5 E	20 (S)	14 (R)	25 (S)	00 (R)

6 E	21 (S)	00 (R)	15 (I)	11 (R)
7 E	18 (S)	24 (S)	28 (S)	13 (R)
8 E	19 (S)	15 (I)	00 (R)	00 (R)
9 E	19 (S)	11 (R)	11 (R)	14 (I)
10 E	12 (R)	00 (R)	14 (R)	14 (I)
11 E	22 (S)	12 (R)	12 (R)	29 (S)
12 E	16 (I)	12 (R)	00 (R)	27 (S)
13 E	15 (I)	23 (S)	29 (S)	25 (S)
14 E	10 (R)	15 (I)	28 (S)	28 (S)
15 E	22 (S)	29 (S)	29 (S)	26 (S)
16 E	20 (S)	13 (R)	18 (I)	16 (I)
17 E	15 (I)	14 (R)	25 (S)	24 (S)
18 E	11 (R)	11 (R)	00 (R)	22 (S)
19 E	20 (S)	26 (S)	24 (S)	22 (S)
20 E	11 (R)	00 (R)	26 (S)	11 (R)
21 E	21 (S)	27 (S)	10 (R)	29 (S)
22 E	14 (R)	00 (R)	22 (S)	27 (S)
23 E	18 (S)	00 (R)	24 (S)	26 (S)

Isolate Code	**Zone of Inhibition**			
	Ceftazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
24 E	18 (S)	12 (R)	23 (S)	24 (S)
25 E	19 (S)	15 (I)	21 (S)	00 (R)
26 E	19 (S)	28 (S)	21 (S)	00 (R)

27 E	19 (S)	11 (R)	00 (R)	12 (R)
28 E	20 (S)	20 (I)	11 (R)	10 (R)

Key:

S: Sensitive

R: Resistant

I: Intermediate

Table 15: Susceptibility of *E-coli* n (28) to

Activity of Antibiotic	Activity of Antibiotic			
	Ceftazidime	**Cefoperazone**	**Cefotaxime**	**Ceftriaxone**
Resistance	7 (25%)	9 (32%)	17 (61%)	10 (36%)
Sensitive	18 (64%)	17 (61%)	7 (25%)	15 (53%)
Intermediate	3 (11%)	2 (7%)	4 (14%)	3 (11%)
Total	28 (100%)	28 (100%)	28 (100%)	28 (100%)

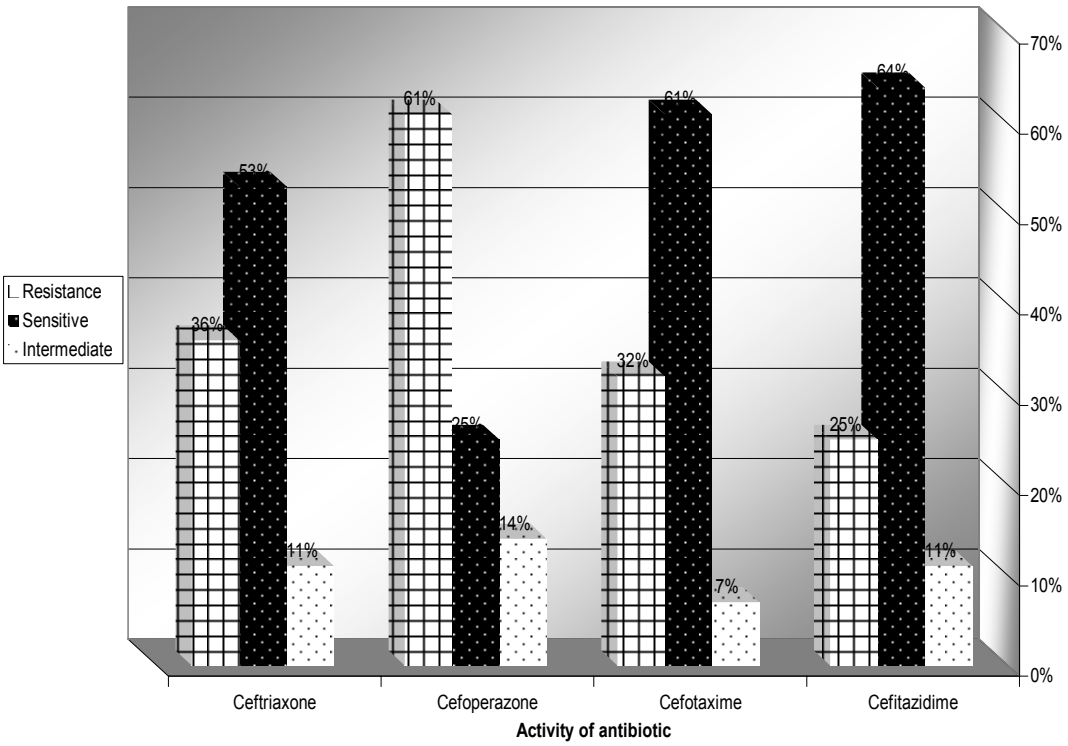

Figure (4): Susceptibility of *E-coli* n (28) to Ceftazidime, Cefoperazone, Cefotaxime & Ceftriaxone

Table 16: Diameters of zone inhibition of antimicrobial (mm) against *Pseudomonase aeruginosa*

Isolate Code	Zone of Inhibition (mm)			
	Ceftazidime	Cefotaxime	Cefoperazone	Ceftriaxone
1 Ps	20 (S)	00 (R)	15 (I)	00 (R)
2 Ps	26 (S)	00 (R)	12 (R)	00 (R)
3 Ps	27 (S)	18 (I)	23 (S)	00 (R)
4 Ps	26 (S)	18 (I)	23 (S)	00 (R)
5 Ps	23 (S)	18 (I)	23 (S)	00 (R)
6 Ps	19 (S)	00 (R)	10 (R)	00 (R)
7 Ps	22 (S)	00 (R)	00 (R)	00 (R)
8 Ps	27 (S)	19 (I)	24 (S)	00 (R)
9 Ps	27 (S)	18 (I)	24 (S)	18 (I)

10 Ps	24 (S)	18 (I)	23 (S)	00 (R)
11 Ps	27 (S)	18 (I)	24 (S)	18 (I)
12 Ps	26 (S)	17 (I)	22 (S)	00 (R)
13 Ps	24 (S)	00 (R)	00 (R)	00 (R)
14 Ps	23 (S)	18 (I)	24 (S)	18 (I)
15 Ps	25 (S)	00 (R)	00 (R)	00 (R)
16 Ps	28 (S)	18 (I)	23 (S)	00 (R)
17 Ps	25 (S)	18 (I)	21 (S)	00 (R)
18 Ps	23 (S)	00 (R)	00 (R)	12 (R)
19 Ps	27 (S)	17 (I)	24 (S)	18 (I)
20 Ps	27 (S)	18 (I)	22 (S)	18 (I)
21 Ps	24 (S)	17 (I)	23 (S)	00 (R)
22 Ps	13 (R)	00 (R)	00 (R)	00 (R)
23 Ps	22 (S)	13 (R)	18 (I)	18 (I)

Isolate Code	**Zone of Inhibition (mm)**			
	Cefitazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
24 Ps	26 (S)	17 (I)	24 (S)	17 (I)
25 Ps	25 (S)	15 (I)	21 (S)	00 (R)
26 Ps	24 (S)	13 (R)	21 (S)	18 (I)
27 Ps	20 (S)	13 (R)	18 (I)	00 (R)
28 Ps	23 (S)	00 (R)	21 (S)	00 (R)
29 Ps	24 (S)	15 (I)	21 (S)	21 (S)
30 Ps	20 (S)	15 (I)	18 (I)	16 (S)
31 Ps	23 (S)	18 (I)	21 (S)	15 (I)
32 Ps	22 (S)	00 (R)	00 (R)	00 (R)

33 Ps	22 (S)	00 (R)	00 (R)	00 (R)
34 Ps	13 (R)	13 (R)	17 (R)	00 (R)
35 Ps	19 (S)	00 (R)	22 (S)	00 (R)
36 Ps	25 (S)	15 (I)	22 (S)	17 (I)
37 Ps	22 (S)	12 (R)	16 (I)	00 (R)
38 Ps	21 (S)	15 (I)	18 (I)	18 (I)
39 Ps	21 (S)	00 (R)	24 (S)	00 (R)
40 Ps	23 (S)	12 (R)	16 (I)	23 (S)
41 Ps	20 (S)	00 (R)	18 (I)	00 (R)
42 Ps	15 (R)	15 (I)	00 (R)	17 (I)
43 Ps	21 (S)	12 (R)	22 (S)	00 (R)
44 Ps	12 (R)	17 (I)	16 (I)	00 (R)
45 Ps	13 (R)	15 (I)	18 (I)	00 (R)

Key:

S: Sensitive

R: Resistant

I: Intermediate

Table 17: Susceptibility of *Ps* n (45) to

Activity of antibiotic	Activity of antibiotic			
	Ceftazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
Resistance	5 (11%)	21 (47%)	11 (25%)	29 (64%)
Sensitive	40 (89%)	-	24 (53%)	4 (9%)
Intermediate	00 (00%)	24 (53%)	10 (22%)	12 (27%)
Total	45 (100%)	45 (100%)	45 (100%)	45 (100%)

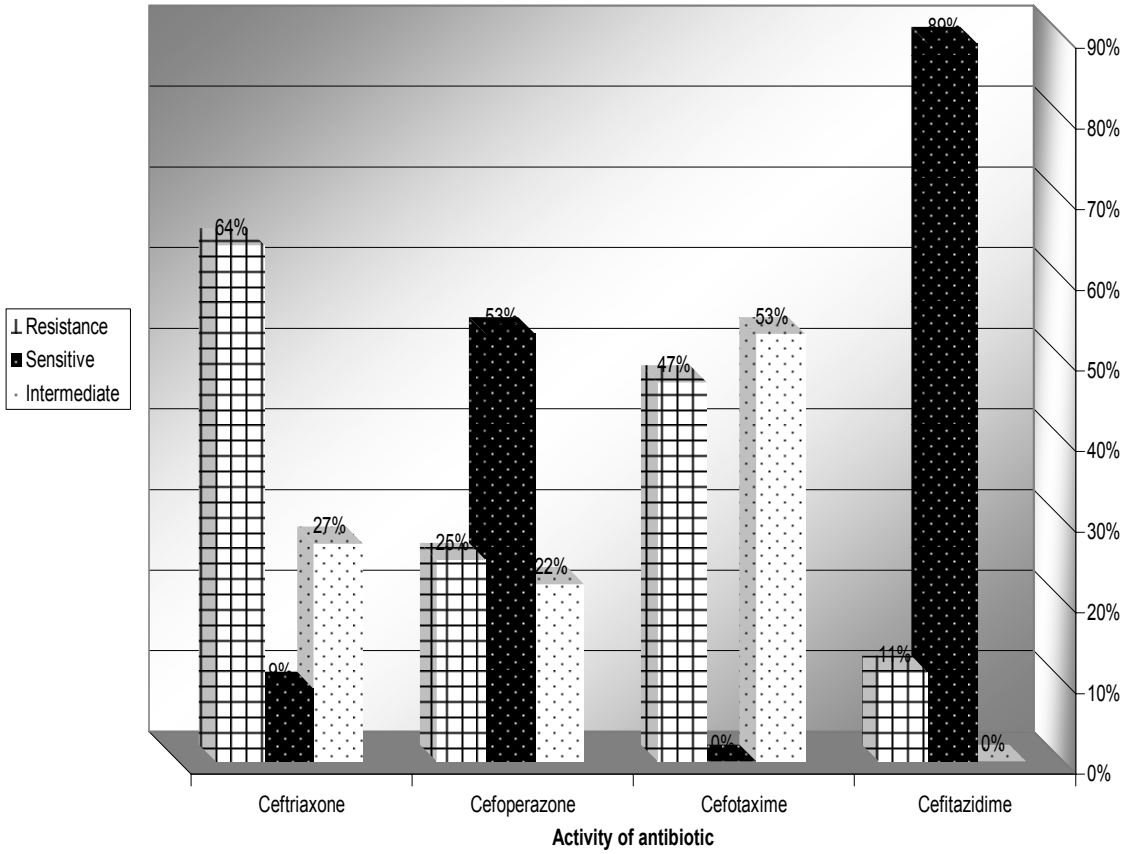

Figure (5): Susceptibility of *Ps* n (45) to Cefitazidime, Cefotaxime, Cefoperazone & Ceftriaxone

Chapter Four

Discussion

Most wound infection probably comes from the patient or personal through direct contact during the operation or later in recovery rooms or wards.

This study was conducted to isolate clinical specimens recovered from infected wound and to test invitro activities of four members of the third generation cephalosporins **(Cefitazime, Cefoperazone, cefotaxime**

and Ceftriaxone) were evaluated using 173 isolates of bacteria by Kirby Bauer disc diffusion method, judged by criteria of National Committee for Clinical Laboratory Standard.

The common causative bacterial gents of wound infections are *staphylococci (55 of all wound infections).*

This result is in alignment with most clinical studies from wound swab in Sudan that showed predominant presence of *Staphylococcus aureus*. Elegail, 1994 found that in wound infection, *Staph .aureus* contributes to 52% of the total samples collected.

The rate of resistance among *Staph aureus* 40 (73%) to Cefitazidime, while the resistance of these isolates to cefoperazone was found 31 (65%).

The other two antibiotics Ceftriaxone 24(44%) and cefotaxime 20 (36%) are less resistance respectively. These results confirm the finding of Bonomo (2003).

The tested isolates 16 (64%) were sensitive to Cefitazidime. The other antibiotics cefoperazone 14 (56%), ceftriaxone 8 (32%) and cefotaxime 6 (24%) were less active on *Proteus Vulgaris*, similar result by Onche and Adedgi (2004).

The total number of *Klebsiella Pneumoniae* isolates was 20, 15 (75%) were sensitive Cefitazidime. While sensitivity of these isolates to cefoperazone 13 (65%). the other two antibiotics Ceftriaxone 11(55%) and cefotaxime 7 (35%) where less active, these result confirm the finding of Onche and Adedgi (2004).

Escherichia coli is sensitive to Ceftazidime 18 (64%), also sensitive to cefoperazone 17 (61%), were less active to ceftriaxone 15(53%) and

cefotaxime 7 (25%). This result near to that obtained by Leonid *et al.*, 1998.

Of the tested isolates 40 (89%) were sensitive to Ceftazidime.While sensitivity of this isolates to cefoperazone was found 24(53%).

The other two antibiotics ceftriaxone and cefotaxime where less active on *Pseudomonase aeruginosa*, their actives were 4(9%) and 00 (00%) respectively.

These results confirm the finding of Massad (2005) who studied the activity of Ceftazidime on *Ps.aeruginosa as (87%)* similar result (89%) and (89%) obtained else where (Kachrid and Ben Hassan, 2000 and Qadri, *et al*., 1991) respectively.

The activity of cefoperazone in the present study was found similar to the result obtained by Sarver, *et al.,*(1981) and Lee *et al., (1990).*

On the other hand, our finding of cefotaxime agrees with those of Bonomo (2003) who reported that cefotaxime was 47%. This result was significantly different from that obtained by Tayseer (1997). Moreover, Watankunakorn (1983) reported that the majority of *Ps.aeruginosa* resistance to cefotaxime.

Isolate code	Zone of Inhibition			
	Ceftazidime	**Cefotaxime**	**Cefoperazone**	**Ceftriaxone**
1 Pr	18 (S)	00 (R)	22 (S)	18 (I)
2 Pr	18 (S)	13 (R)	21 (S)	00 (R)
3 Pr	20 (S)	10 (R)	24 (S)	12 (R)
4 Pr	14 (R)	00 (R)	26 (S)	13 (R)
5 Pr	22 (S)	14 (R)	24 (S)	22 (S)
6 Pr	22 (S)	00 (R)	00 (R)	15 (I)
7 Pr	13 (R)	16 (I)	16 (I)	26 (S)
8 Pr	23 (S)	24 (S)	13 (R)	28 (S)
9 Pr	23 (S)	24 (S)	29 (S)	30 (S)
10pr	19 (S)	13 (R)	12 (R)	25 (S)
11 Pr	18 (S)	11 (R)	00 (R)	12 (R)
12 Pr	22 (S)	11 (R)	25 (S)	00 (R)
13 Pr	21 (S)	12 (R)	11 (R)	13 (R)
14 Pr	12 (R)	15 (I)	24 (S)	14 (I)
15 Pr	17 (I)	26 (S)	28 (S)	32 (S)
16 Pr	17 (I)	25 (S)	00 (R)	16 (I)
17 Pr	22 (S)	00 (R)	10 (R)	24 (S)
18 Pr	22 (S)	11 (R)	00 (R)	20 (I)
19 Pr	16 (I)	18 (I)	25 (S)	23 (S)
20 Pr	11 (R)	13 (R)	14 (R)	00 (R)
21 Pr	11 (R)	26 (S)	21 (S)	10 (R)
22 Pr	16 (I)	00 (R)	11 (R)	11 (R)
23 Pr	20 (S)	19 (I)	29 (S)	00 (R)
24 Pr	22 (S)	10 (R)	25 (S)	00 (R)
25 Pr	20 (S)	23 (S)	24 (S)	10 (R)

Key:

S: Sensitive

R: Resistant

I: Intermediate

Table 11: Susceptibility of *Proteus vulgaris* n (25) to

Activity of Antibiotic	Activity of Antibiotics			
	Ceftazidime	**Cefoperazone**	**Cefotaxime**	**Ceftriaxone**
Resistant	5 (20%)	10 (40%)	15 (60%)	12 (48%)
Sensitive	16 (64%)	14 (56%)	6 (24%)	8 (32%)
Intermediate	4 (16%)	1 (4%)	4 (16%)	5 (20%)
Total	25 (100%)	25 (100%)	25 (100%)	25 (100%)

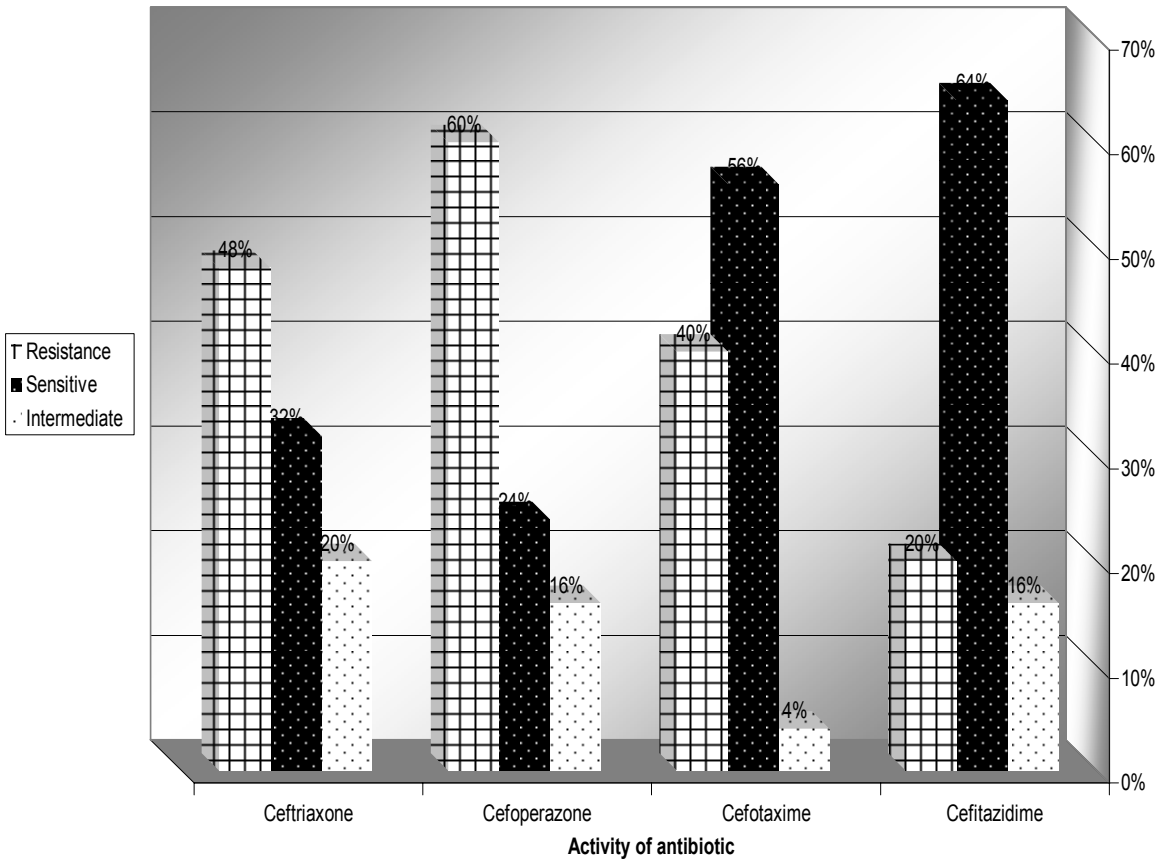

Figure (2):Susceptibility of *Pr* n (25) to Ceftazidime, Cefoperazone, Cefotaxime & Ceftriaxone